Disproportionate dwarf and eye defect (*dde*): A new recessive mutation with eye defects has been found on Chromosome 1.

Belinda S. Harris, Patricia F. Ward-Bailey, David E. Bergstrom, Muriel T. Davisson-Fahey, and Roderick T. Bronson

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Mutation (allele) symbol: *dde*

Mutation (allele) name: disproportionate dwarf and eye defect

Gene symbol: *dde*

Strain of origin: CByJ.Cg-*hop*/J

Current strain name: CByJ(Cg)-*dde*/GrsrJ

Stock #010822 (jaxmice.jax.org)

Phenotype categories: skeletal, eye

Origin and Description

This spontaneous mutation arose in the year 2000 in the Mouse Mutant Resource at The Jackson Laboratory in a research colony of CByJ.Cg-*hop*/J mice at generation N5F1 (Stock #002718). Mice homozygous for the *dde* mutation can be recognized at birth by their shortened limbs (disproportionate dwarfing) and fused toes in the back feet. It has also been discovered that these mice have a cosegregating eye defect (clinically noted as having white lines in the retina). The CByJ(Cg)-*dde*/GrsrJ strain is maintained by homozygous female x heterozygous male matings when possible. Male homozygotes are usually sterile and female homozygotes may not breed well or take good care of their progeny, so fostering pups to a foster mother may be necessary to maintain the colony. Homozygous female and male *dde* mice live a somewhat varied lifespan; some may live to just past weaning and some may get sickly by one year of age and die. Homozygous *dde* mice need to be housed with parents longer than the usual three weeks of age because of their small size. In addition to the normal grain, ground meal is usually added to their cage to help them thrive.

Genetic Analysis

Using the standard mapping protocols of The Mouse Mutant Resource, a linkage cross was established by breeding two female homozygous *dde* mice to a male CAST/EiJ (*Mus castaneus*) mouse. No *dde* mutants were seen in the F1 progeny from this mating. The F1s from this cross were then intercrossed and mice affected with the *dde* mutation were seen the F2 generation progeny. The F1 intercross produced 52 affected F2 mice of which 42 were used for linkage analysis. Genomic tail DNA preparations were made from the tails of the affected F2 mice and standard PCR methods were used to map the *dde* mutation to Chromosome 1. PCR analysis of a DNA pool of equal aliquots from 21 F2 mutants from the CAST/Ei intercross revealed a deficiency of CAST/Ei specific products for Chromosome 1 with *D1Mit46* (NCBI 37 position 75.5 Mb). The individual

DNA samples from 42 F2 affected animals were then typed with *D1Mit46* and 4 other Chr 1 Mit markers. Combining all data, we have determined that the *dde* mutation maps between *D1Mit251* (NCBI 37 position 70.4 Mb) and *D1Mit162* (NCBI 37 position 76.7 Mb) and is non-recombinant with *D1Mit7* (NCBI 37 position 74.9 Mb). Based on chromosomal position and phenotypic similarities, the Indian hedgehog (*Ihh*) gene (NCBI 37 position 74.9 Mb) is considered to be a good candidate gene.

Pathology

Our standard pathological screen[1] showed that homozygotes may exhibit a variety of lesions: emphysema, stomach polyp, apoptotic cells of the testis, thyroid cysts, holes in the thoracic cord white matter and in the skeletal muscle fibers in the leg. Some animals have been shown to have no corpus callosum, a CByJ.Cg-*hop*/J background strain effect, not caused by the *dde* mutation. X-Ray analysis show that the axial skeleton looks normal but the femur and humerus are severely shortened. This new mutation is a good example of an achondroplastic dwarf with arrested growth plates in the long bones.

The eyes of the mice produced for the linkage cross were examined with an ophthalmoscope and electroretinograms (ERG) were performed. It was determined that +/? mice have normal retinas while homozygotes have abnormalities. Although homozygotes were generally normal when examined with an ophthalmoscope, they had abnormal ERGs with specific abnormalities of the rods and cones. Histology of the eye shows both small and large regions of retinal dysplasia with the large regions of dysplasia appearing clinically as white lines. The eye phenotype was concordant with the leg phenotype in all mice tested. It is a possibility that *dde* mice could be a model for Walker-Wardberg syndrome in humans, but this has not been proven.

Hearing as assessed by auditory brainstem testing (ABR) in all tested mutant and control mice was normal.

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[1]**Standard Histology Protocol used in The Mouse Mutant Resource**

For fixation of tissues, mice were deeply anesthetized with tribromoethanol (avertin) until they no longer displayed a withdrawal reflex in the hind limbs and then perfused intracardially with Bouin's fixative following a flush of the vasculature with saline solution. After soaking in Bouin's for one week to demineralize bones, tissues were dissected. Six segments of spine with axial muscles and spinal cord in situ, representing cervical, thoracic and lumbar spinal segments, were dissected. The brain was removed and sliced into 6 cross sectional pieces at the levels of olfactory lobes, frontal cortex, striatum, thalamus, midbrain, rostral and caudal medulla with cerebellum. Midsagittal slices of hind leg through the knees were prepared. Slices of basal skull through the pituitary and inner ears were taken. Both eyes, salivary glands and submandibular lymph node, trachea plus thyroid and sometimes parathyroid were removed and cassetted. A longitudinal slice of skin from the back was removed. The thymus, slices of lung, and a longitudinal slice of heart were cassetted. Similarly slices of liver through gall bladder, kidney with adrenal attached, pancreas and spleen were prepared. The stomach was sliced longitudinally to include both squamous and glandular portions. Loops of small intestine from 3 levels and slices of large intestine and cecum were removed, as were slices of urinary bladder. The whole uterus, with ovaries attached, was taken. In males testes were sliced longitudinally. The accessory male organs including seminal vesicles,

coagulating gland and prostate were removed en block. Altogether in most cases all tissue fit into a total of 10 cassettes. The cassettes were processed in an automatic tissue processor to dehydrate tissues which were then embedded in paraffin. Six micron sections were cut and stained with hematoxylin and eosin (H&E). Sections of brain and spinal cord in vertebral bones also were stained with luxol fast blue (LFB) for myelin and cresylecht violet (CV) for cellular detail.